

# Wide geographic distribution of *Cryptosporidium bovis* and the deer-like genotype in bovines<sup>☆</sup>

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## Abstract

Recent studies in the United States reported that ~85% of pre-weaned dairy calves were infected with zoonotic *Cryptosporidium parvum*, whereas only 1–2% of post-weaned calves and 1–2-year-old heifers were infected with this species. *Cryptosporidium bovis* and *Cryptosporidium* deer-like genotype were much more prevalent in the post-weaned animals. It is not clear whether the same infection pattern also occurs in other geographic areas. In this study, to determine whether the same *Cryptosporidium* infection pattern was present in other geographic areas, we genotyped *Cryptosporidium* specimens collected from two farms in China and India, using specimens from farms in Georgia, USA for comparison. *C. bovis* was the most common species found in pre- and post-weaned calves in all three areas. In Georgia, the deer-like genotype was found frequently in pre- and post-weaned calves and *Cryptosporidium andersoni* was found in one post-weaned calf. Both *C. bovis* and the deer-like genotype were found in the few milking cows examined in Georgia. There were no differences in the small subunit rRNA gene sequences obtained from *C. bovis* or deer-like genotype among the three areas. One adult yak in China, however, was infected with a species similar to *C. bovis*, with only three nucleotide mutations in the target gene. All four common bovine *Cryptosporidium* spp. were differentiated from each other by restriction fragment length polymorphism analysis of PCR products with enzymes *SspI* and *MboII*. Thus, both *C. bovis* and the deer-like genotype are found in all age groups of cattle in diverse geographic areas and host adaptation of *C. bovis* might have occurred in yaks.

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**Keywords:** *Cryptosporidium*; Calves; Genotyping; Diagnosis; Epidemiology

## 1. Introduction

Cryptosporidiosis is a common protozoan infection in cattle. In addition to causing morbidity and mortality in cattle, bovine cryptosporidiosis is also an important source of human *Cryptosporidium* infections. Contact with infected calves has been implicated as the cause of many cryptosporidiosis outbreaks in veterinary students, research technicians and children attending agricultural

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camps and fairs (Corbett-Feeney, 1987; Current et al., 1983; Kiang et al., 2006; Levine et al., 1988; Miron et al., 1991; Pohjola et al., 1986; Preiser et al., 2003; Reif et al., 1989; Smith et al., 2004). Contamination of food or water by cattle manure has also been identified as a cause of several foodborne and waterborne outbreaks of cryptosporidiosis (Blackburn et al., 2006; Glaberman et al., 2002; Millard et al., 1994). Case control studies conducted in the United States, United Kingdom, Ireland and Australia have implicated contact with cattle as a risk factor in sporadic cases of human cryptosporidiosis (Corbett-Feeney, 1987; Goh et al., 2004; Hunter et al., 2004; Robertson et al., 2002; Roy et al., 2004). Indeed, massive slaughtering of farm animals and restriction of farm visit during foot-and-mouth disease outbreaks reduced sporadic human *C. parvum* infections in large communities (Hunter et al., 2003; Smerdon et al., 2003).

Recent studies in the United States suggest that cattle are infected with at least four *Cryptosporidium* parasites: *C. parvum*, *Cryptosporidium bovis*, *Cryptosporidium andersoni* and the *Cryptosporidium* deer-like genotype (Fayer et al., 2006; Santin et al., 2004; Xiao et al., 2004). The occurrence of these *Cryptosporidium* spp. in cattle were shown to be age-related (Fayer et al., 2006; Santin et al., 2004). *C. parvum*, the only prevalent zoonotic species in cattle, is responsible for about 85% of the *Cryptosporidium* infections in pre-weaned calves but only 1% of the *Cryptosporidium* infections in post-weaned calves and heifers. Post-weaned calves and 1–2-year-old heifers are mostly infected with *C. bovis*, *C. andersoni* and the *Cryptosporidium* deer-like genotype (Fayer et al., 2006; Santin et al., 2004). These findings clearly demonstrate that in the United States only pre-weaned calves are importance sources of zoonotic cryptosporidiosis in humans (Fayer et al., 2006).

Currently, *C. bovis* and the deer-like genotype have been identified in cattle only in the United States (Fayer et al., 2006; Santin et al., 2004; Xiao et al., 2002). It is not clear whether they also occur in cattle in other countries. In this study, to determine whether the same *Cryptosporidium* infection pattern was present in other geographic areas, we genotyped *Cryptosporidium* specimens collected from calves on two farms in China and India, using specimens from farms in Georgia, USA for comparison.

## 2. Materials and methods

### 2.1. Specimens

A total of 50 *Cryptosporidium*-positive fecal specimens from dairy cattle (*Bos taurus*) were

included in this study. They included six specimens collected from calves (five 1–5-week-old calves and one 3-month-old calf) on a farm in Shanghai, China and 12 from calves (six 1-month-old calves and six 2–6-month-old calves) on a farm in Kolkata, India. As a comparison, 32 specimens collected from three farms in Georgia, USA were also used, including 23 from pre-weaned calves of under 8 weeks, six from post-weaned calves of 8 weeks to 8 months and three from milking cows. All specimens were collected from dairy cattle in the three countries during 2004 and 2005. In addition, one *Cryptosporidium*-positive specimen from an 8-year-old yak in China was also studied.

### 2.2. DNA extraction and conventional genotyping

Genomic DNA was extracted from *Cryptosporidium*-positive specimens by alkaline digestion and phenol–chloroform extraction, followed by DNA purification using the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, Calif.), as previously described (Peng et al., 2003). *Cryptosporidium* spp. were genotyped initially by nested PCR amplification of an approximately 830 bp fragment of the small subunit (SSU) rRNA gene and restriction fragment length polymorphism (RFLP) analysis of the secondary PCR products using restriction enzymes *SspI* and *VspI* (Xiao et al., 2001). All secondary PCR products were sequenced to confirm the genotype identification. Each specimen was analyzed at least twice by the PCR-RFLP technique. DNA of *C. serpentis* was used as the positive control in most analyses and reagent water was used as negative control in all SSU rRNA-based PCR-RFLP analysis.

### 2.3. Genotyping using *MboII* RFLP analysis

To develop a RFLP method for the rapid differentiation of *C. parvum*, *C. bovis* and the deer-like genotype, SSU rRNA gene sequences from the three species were searched for restriction enzyme cutting sites using the software BioEdit ([www.mbio.ncsu.edu/BioEdit/bioedit.html](http://www.mbio.ncsu.edu/BioEdit/bioedit.html)). The restriction maps that were generated were manually compared and the restriction enzyme generating genotype-unique pattern, *MboII*, was chosen. Ten microliters of the secondary PCR products of the SSU rRNA gene were digested with *MboII* (New England BioLabs, Beverly, MA) per conditions suggested by the manufacturer. The restriction digestion products were separate by electrophoresis on 2% agarose gels.

## 2.4. Detection and subtyping of *C. parvum*

To detect light *C. parvum* infection in the dominance of other *Cryptosporidium* spp. and to subtype *C. parvum*, all specimens were also analyzed by a nested PCR targeting the 60 kDa glycoprotein (GP60) gene (Alves et al., 2003). This method amplifies an approximately 850 bp fragment of the GP60 gene of *C. parvum* and related species, but does not amplify DNA of *C. bovis*, *C. andersoni* and *Cryptosporidium* deer-like genotype. Subtypes were determined and named based on both the number of trinucleotide repeats and mutations in the non-repeat regions (Sulaiman et al., 2005).

## 2.5. DNA sequence analysis

All positive PCR products generated in the study were sequenced, using the forward and reverse primer used in secondary PCR. GP60 PCR products were further sequenced with an intermediary sequencing primer (5'-GAGATATATCTTGTTCG-3'). Sequencing reactions were done using purified products and the ABI BigDye Terminator Version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), following the manufacturer's instructions. Sequences were read on an ABI3100 Genetic Analyzer (Applied Biosystems). The nucleotide sequences obtained in this study were aligned with reference sequences retrieved from the GenBank using the program ClustalX (<ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/>). Unique nucleotide sequences generated in the study were deposited in the GenBank database under accession numbers DQ871345–DQ871350.

## 3. Results

### 3.1. *Cryptosporidium* genotypes by the conventional PCR-RFLP tool

All *Cryptosporidium*-positive specimens generated the expected SSU rRNA gene products in nested PCR. Restriction analysis produced similar *SspI* banding patterns and identical *VspI* banding pattern for all PCR products, with the exception of one specimen from a post-weaned 62-day-old calf in Georgia, which produced a banding pattern unique for *C. andersoni*. The *SspI* RFLP pattern of most PCR products was similar to that of *C. parvum*. However, PCR products of only six specimens yielded the *SspI* RFLP pattern identical to *C. parvum* (three visible bands of 449, 267 and 108 bp). Some *SspI* RFLP products yielded an upper band

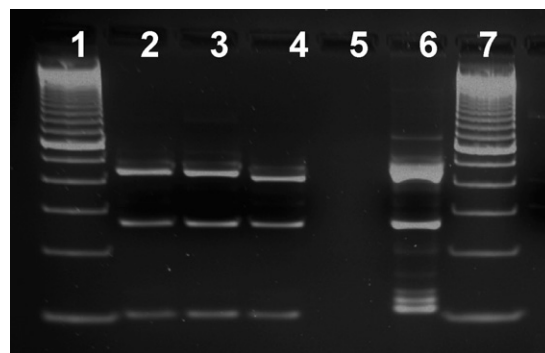


Fig. 1. Similarity in *SspI* RFLP patterns of the SSU rRNA gene PCR products among *C. parvum*, *C. bovis* and the deer-like genotype in cattle and one *C. bovis*-like parasite in a yak. (Lanes 1 and 7) 100 bp molecular markers; (lane 2) *Cryptosporidium* deer-like genotype; (lane 3) *C. bovis*; (lane 4) *Cryptosporidium* in a yak; (lane 5) negative control; (lane 6) *C. parvum* (positive control). The lower doublet bands in *C. parvum* were due to the presence of both A and B copies of the SSU rRNA gene products in the PCR products.

(432 bp) and a lower band (103 bp) that were slightly smaller than those of *C. parvum* (Fig. 1 and Table 1), whereas other PCR products had a combination of the regular and the smaller upper bands (Fig. 2). The *SspI* RFLP pattern of the yak isolate had an upper *SspI* band (413 bp) that was significantly smaller than the one seen in other PCR products (Fig. 1 and Table 1).

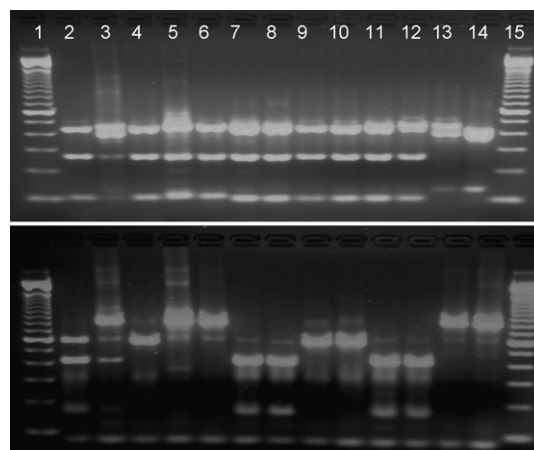


Fig. 2. Differentiation of four common bovine *Cryptosporidium* species by *SspI* (upper panel) and *MboII* (lower panel) RFLP analyses of the SSU rRNA gene PCR products. (Lanes 1 and 15) 100 bp molecular markers; (lane 2) *C. bovis* and the deer-like genotype; (lane 3) *C. andersoni* with traces of *C. bovis*; (lanes 4, 9 and 10) the deer-like genotype; (lanes 5 and 6) *C. parvum*; (lanes 7, 8, 11 and 12) *C. bovis*; (lane 13) *C. andersoni*; (lane 14) positive control (*C. serpentis*). Lanes 2 and 4 are different products of one specimen (11070) and lanes 3, 6 and 13 are different products of another specimen (11084). The partial digestion product of 574 bp is seen in most lanes.

Table 1

Differentiation of *C. parvum*, *C. bovis*, *C. andersoni* and *Cryptosporidium* deer-like genotype by RFLP analysis with *SspI*, *VspI* and *MboII*

<i>Cryptosporidium</i>	PCR product size (bp)	<i>SspI</i> products (bp)	<i>VspI</i> products (bp)	<i>MboII</i> products (bp)
<i>C. parvum</i>	847	449, 267, 108, 12 <sup>a</sup> , 11 <sup>a</sup>	628, 115, 104	771, 76
<i>C. bovis</i>	835	432, 267, 103, 33 <sup>a</sup>	616, 115, 104	412, 185 <sup>a</sup> , 162, 76
<i>C. bovis</i> in yak	835	413, 267, 103, 33 <sup>a</sup> , 19 <sup>a</sup>	616, 115, 104	412, 185 <sup>a</sup> , 162, 76
Deer-like genotype	835	432, 267, 103, 33 <sup>a</sup>	616, 115, 104	574, 185 <sup>a</sup> , 76
<i>C. andersoni</i>	845	448, 397	730, 115	769, 76

<sup>a</sup> Not visible in agarose gel electrophoresis.

### 3.2. *Cryptosporidium* genotypes by DNA sequencing

DNA sequencing of PCR products confirmed the identification of *C. andersoni* in the 62-day-old post-weaned calf from Georgia. It also confirmed that PCR products with identical *C. parvum* *SspI* pattern to be *C. parvum*. In contrast, SSU rRNA gene products with smaller *SspI* upper and lower bands were from either *C. bovis* or the *Cryptosporidium* deer-like genotype, whereas those with a combination of the regular and the smaller upper bands belonged to *C. bovis* based on results of DNA sequencing. Altogether, *C. parvum* was identified in only six specimens collected from pre-weaned calves in Georgia and one pre-weaned calf specimen collected in India and the deer-like genotype was identified in five pre-weaned calves and one post-weaned calf in Georgia and one pre-weaned calf in China. In contrast, *C. bovis* was the most commonly detected *Cryptosporidium*, being found in nine pre-weaned calves, four post-weaned calves and two milking cows in Georgia, five pre- and post-weaned calves in China and 11 pre- and post-weaned calves in India (Table 2). The specimen with *C. andersoni* collected from a post-weaned calf on a Georgia farm also had concurrent *C. parvum* infection and possibly a *C. bovis* infection (Fig. 2). In addition, three pre-weaned calves and one milking cow in Georgia had concurrent infections of *C. bovis* and the deer-like genotype as indicated by DNA sequencing of multiple PCR products (Fig. 2). The SSU rRNA sequences of *C. andersoni*, *C. parvum*, *C. bovis* and the deer-like genotype were identical to those previously reported in the United States (Santin et al., 2004; Xiao et al., 2002). The sequence obtained from the *Cryptosporidium* of the adult yak in China, however, had three nucleotide differences from that of *C. bovis*. One of the nucleotide changes in the yak sequence produced a new *SspI* restriction site (Fig. 3).

### 3.3. Differentiation of *C. parvum*, *C. bovis* and deer-like genotype by *MboII* RFLP

SSU rRNA sequences of *C. andersoni*, *C. bovis*, *C. parvum* and the deer-like genotype were searched for restriction enzyme cutting sites using software BioEdit. This led to the identification of the restriction enzyme *MboII* for the differentiation of *C. bovis*, *C. parvum* and the deer-like genotype, which was difficult using the conventional *SspI* and *VspI* restriction (Table 1). In theory, *MboII* digestion of the secondary SSU rRNA PCR products would generate two bands for *C. parvum* (76 and 771 bp), three bands for the deer-like genotype (76, 185 and 574 bp) and four bands for *C. bovis* (76, 162, 185 and 412 bp). *Cryptosporidium andersoni* would also produce two bands (76 and 769 bp), but it could be easily differentiated from *C. parvum* by *SspI* restriction (Fig. 2 and Table 1). All positive SSU rRNA PCR products generated in the study were digested with *MboII* and the expected banding patterns were generated for *C. parvum* and *C. andersoni*. The expected 185 bp band was not seen for both *C. bovis* and deer-like genotype, but this did not affect the differentiation of these two and *C. parvum* (Fig. 2). Results of *MboII* restriction also supported the identification of mixed infections with *C. bovis* and the deer-like genotype in three pre-weaned calves and one milking cow in Georgia (Table 2 and Fig. 2).

### 3.4. Detection and subtyping of *Cryptosporidium* by GP60 PCR

All specimens were also analyzed by GP60 PCR irrespective of the SSU rRNA genotyping results. Six specimens from pre-weaned calves in Georgia were positive, including those from four and two calves that were positive for *C. parvum* and *C. bovis* by SSU rRNA-based PCR, respectively. Nine of the 12 specimens from pre- and post-weaned calves in India were also positive in GP60 PCR, including one and eight animals that were positive for *C. parvum* and *C. bovis* by SSU rRNA-

Table 2

Detection of *Cryptosporidium* genotypes by SSU rRNA-based PCR-RFLP and *C. parvum* and *C. hominis* subtypes by GP60-based PCR-sequencing in bovine specimens collected in three different areas

Source	Age group	No. sample	SSU rRNA PCR-RFLP result					GP60 PCR result	
			<i>C. parvum</i>	<i>C. bovis</i>	Deer-like genotype	<i>C. bovis</i> + deer-like	<i>C. parvum</i> + <i>C. andersoni</i>	No. positive	Subtype (no. of specimens)
Georgia	Pre-weaned calves	23	6	9	5	3	0	6 (including 4 <i>C. parvum</i> specimens and 2 <i>bovis</i> specimens)	IlaA15G2R1 (6)
	Post-weaned calves	6	0	4	1	0	1 <sup>a</sup>	0	–
	Milking cows	3	0	2	0	1	0	0	–
China	Pre- and post-weaned calves	6	0	5	1			0	–
India	Pre- and post-weaned calves	12	1	11	0	0	0	9 (including 1 <i>C. parvum</i> specimen and 8 <i>C. bovis</i> specimens)	IlaA15G2R1 (5), IlaA13G2R2 (1), IlaA14G2R1a (1), IlaA14G2R1b (1) <sup>a</sup> and IdA15G1 (1) <sup>b</sup>

<sup>a</sup> Having one nucleotide difference (a change of A to G) from IlaA14G2R1a downstream of the trinucleotide repeats.

<sup>b</sup> A *C. hominis* subtype. All other GP60 subtypes were of *C. parvum*.



<i>C. bovis</i>	AATATTTTGGTGACTCATAATAACTTTACGGATCACATTATGTGACATATCATTCAAGTTTCTGACCTA
Yak	.....
Deer-like	.....C.....
<i>C. bovis</i>	TCAGCTTTAGACGGTAGGGTATTGGCCTACCGTGGCTATGACGGGTAACGGGGAATTAGGGTTCGATTCC
Yak	.....
Deer-like	.....
<i>C. bovis</i>	GGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTAA
Yak	.....
Deer-like	.....
<i>C. bovis</i>	TACAGGGAGGTAGTGACAAGAAATAACAATACAGAACCTTACGGTTTTGTAATTGGAATGAGTTAAGTAT
Yak	.....
Deer-like	.....G.....
<i>C. bovis</i>	AAACCCCTTAACAAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATA
Yak	.....
Deer-like	.....
<i>C. bovis</i>	GCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTAATCTTCTGTTAATTTTATATATAATATCA
Yak	.....TT
Deer-like	.....T.....C...GCT.
<i>C. bovis</i>	CGATATTTATATAATATTAACATAATTCATATTACTTTTGTAGTATATGAACTTTACTTTGAGAAAATTA
Yak	.....
Deer-like	..G.....
<i>C. bovis</i>	GAGTGCTTAAAGCAGGCTATTGCCTTGAATACTCCAGCATGGAATAATATTAAGGATTTTATTCTTCTT
Yak	.....
Deer-like	.....
<i>C. bovis</i>	ATTGGTTCTAGAATAAAAAATGATGATTAATAGGGACAGTTGGGGGCATTGTATTTAACAGTCAGAGGTG
Yak	.....A.....
Deer-like	.....A.....

Fig. 3. Differences in the 835 bp fragment of the SSU rRNA gene among *C. bovis*, the deer-like genotype and a *C. bovis*-like parasite in a yak. Dots denote nucleotide identity to the *C. bovis* sequence. Only the sequences of the polymorphic region are shown. Comparing to *C. bovis*, there are three nucleotide changes in the yak sequence, one of which has led to the creation of a new *SspI* restriction site (underlined).

based PCR, respectively. None of the specimens collected from six pre- and post-weaned calves in China were positive in GP60 PCR (Table 2).

DNA sequencing indicated that all six GP60 positive specimens from pre-weaned calves in Georgia had the *C. parvum* subtype IIaA15G2R1. Five of the nine positive pre- and post-weaned calves in India also had IIaA15G2R1. In contrast, the other four Indian calves had *C. parvum* subtypes IIaA13G2R2, IIaA14G2R1a and IIaA14G2R1b and the *Cryptosporidium hominis* subtype IdA15G1 (Table 2). The *C. parvum* subtypes differed from each other mostly in the trinucleotide repeat region. However, subtypes IIaA14G2R1a and IIaA14G2R1b differed from each other only with an A to G change downstream of the trinucleotide repeats. The identification of the *C. hominis* subtype IdA15G1 in one post-weaned Indian calf was confirmed in two subsequent PCR and sequencing runs by another investigator in the laboratory.

#### 4. Discussion

Results of this study clearly demonstrate that *C. bovis* and the deer-like genotype also infect cattle in countries in addition to the United States. In addition to cattle in Georgia and in seven other states where they were previously detected (Santin et al., 2004; Fayer et al., 2006), *C. bovis* and the deer-like genotype were found in the small number of cattle studied in China and India. The prevalence of these two species was much higher than that of *C. parvum*, even in pre-weaned calves in Georgia. This was probably largely due to the fact that pre-weaned calves examined in this study were mostly 1 month or older and only a small number of animals were sampled. Previously, it was shown that these two parasites were mostly found in 15% of pre-weaned dairy calves, 85% of post-weaned dairy calves and 48% of 1–2-year-old heifers in the United States (Fayer et al., 2006; Santin et al., 2004). Thus, the earlier

suggestion that pre-weaned calves are the most significant source of zoonotic infection probably also applies to other countries.

The reason for the previous observation on the age-related occurrence of *Cryptosporidium* genotypes is not clear. The common identification of *C. bovis* in pre-weaned calves in this study suggests that calves may acquire infection with *C. bovis* or the deer-like genotype early in life, but the infection might have been concealed by the overwhelming *C. parvum* infection. The prepatent period for *C. bovis* is 10–12 days, much longer than *C. parvum* (about 4 days) (Fayer et al., 2005). Because the intensity of oocyst shedding associated with *C. parvum* is at least four logs higher than that associated with presumed infections with *C. bovis* and the deer-like genotype (Xiao and Herd, 1994), the light infection with the latter species in pre-weaned calves would be very difficult to detect. The frequent concurrent *C. parvum* and *C. bovis* infections in older calves observed in this study further supports the conclusion. Previously, it was shown that SSU rRNA-based PCR tools selectively amplify the predominant *Cryptosporidium* species (Cama et al., 2006; Reed et al., 2002). In the present study, the detection of light *C. parvum* infection in older calves in the presence of *C. bovis* was made possible by using a PCR tool that selectively amplifies *C. parvum* and a few *C. parvum*-related species. Thus, even if *C. bovis* is present in *C. parvum*-infected pre-weaned calves, the less specific SSU rRNA-based PCR tool would have failed to detect it.

The detection of *C. bovis* infections and the deer-like genotype in pre- and post-weaned calves and adult cattle in this study suggests that cattle of all ages are susceptible to infections with both species and there may not be an age-associated occurrence of the two parasites in cattle. In this study, *C. bovis* infection was detected as early as 2-weeks-of-age in a calf in China and 3-weeks-of-age in two calves in China and Georgia and as late as two milking cows in Georgia and an 8-year-old yak in China. The deer-like genotype was also detected in both pre- and post-weaned calves and in milking cows. This is in agreement with the recent report of frequent finding of the two species in 1–2-year-old cattle in the United States (Fayer et al., 2006). In this study and two previous studies conducted in the United States, the host spectrum of *C. bovis* and the deer-like genotype is similar, even though the prevalence of the infections with the deer-like genotype is generally lower than that of *C. bovis*. This is not surprising considering the genetic relatedness of the two *Cryptosporidium* species (Xiao et al., 2004).

The lack of finding of *C. bovis* and the deer-like genotype in previous molecular epidemiologic studies is probably partially due to the use of older genotyping tools. Previously, the detection of *Cryptosporidium* genotypes in cattle was made mostly using PCR-RFLP tools based on the SSU rRNA gene and PCR-RFLP or PCR-sequencing tools based on protein genes or unknown genomic fragments (Alves et al., 2003; Glaberman et al., 2002; Huetink et al., 2001; Learmonth et al., 2003; Mallon et al., 2003; Peng et al., 2003; Peng et al., 1997; Trotz-Williams et al., 2006). Because the latter were largely developed using sequences of *C. parvum*, they are unlikely to amplify DNA from the distant *C. bovis* and deer-like genotype based on experiences with other *Cryptosporidium* spp. in humans (Jiang and Xiao, 2003). Because there are only minor differences in RFLP patterns among *C. parvum*, *C. bovis* and the deer-like genotype in SSU rRNA-based PCR tools, the reliable differentiation of the three species generally requires the DNA sequencing of the PCR products (Fayer et al., 2006; Santin et al., 2004). In the present study, a new restriction enzyme *Mbo*II is used for the easy differentiation of these three *Cryptosporidium* species. Its use in conjunction with *Ssp*I RFLP should facilitate rapid genotyping of all four common *Cryptosporidium* spp. in cattle (Fig. 2).

In this study, a *C. bovis*-like parasite was identified in an adult yak. This parasite had only three nucleotide changes in the targeted fragment of the SSU rRNA gene and was related to *C. bovis* and the deer-like genotypes in cattle and the deer genotype in deer. These parasites represent a group of closely related but host-adapted *Cryptosporidium* spp. in ruminants. It is likely more members will be found when parasites of other wild and domesticated ruminants are characterized.

The *C. parvum* GP60 subtype IIaA15G2R1 was commonly found in calves in both Georgia and India. This subtype was previously shown to be the most prevalent *C. parvum* subtype in calves and humans in the United States, Canada, United Kingdom, Portugal, Australia, Japan and Kuwait (Abe et al., 2006; Alves et al., 2003, 2006; Chalmers et al., 2005; Peng et al., 2003; Sulaiman et al., 2005; Trotz-Williams et al., 2006; Wu et al., 2003). Three other *C. parvum* subtypes were also found in the few calves studied in India, including IIaA13G2R2, IIaA14G2R1a and IIaA14G2R1b. The latter, however, have not been seen so far in other areas. One post-weaned calf in India had a light infection of *C. hominis* in the presence of *C. bovis*. *Cryptosporidium hominis* infection, sometimes concurrently with *C. parvum*, has been found previously in a few calves in the United States and Scotland (Smith et al., 2005;

Tanriverdi et al., 2003). The *C. hominis* subtype (IdA15G1) found in the Indian calf was also recently found in children in the study area (Kolkata) (data not shown), suggesting that humans might have transmitted the infection to the study calf.

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